CHAPTER 3.1

DOSIMETRIC STUDIES ON DETERRENT ACTION OF LEAF EXTRACTS OF 5 PLANTS ON ADULT AND LATE LARVAL INSTARS OF CIGARETTE BEETLE, *LASIODERMA SERRICORNE* F.

Majority of the insects are either harmless or beneficial to human beings, but only about 1 percent of the insects are harmful by way of spreading diseases, destroying crops as well as stored grains. The artificial application of heat has long been recognised as one of the methods of choice in controlling pests of stored products (Goodwin, 1914; Hurst and Black, 1930; Grossman, 1931; Zacher, 1935; Pruthi and Singh, 1950 and Cotton, 1960). In the recent past, man has invented many highly potent organic chemicals for the control of such harmful insect pests. However, these agents adversly affect the natural ecological balance and also harm to man and his live-stocks due to residual activity. Now a days, therefore, increasing emphasis is being given on use of naturally occuring pesticides of plant origins which are safer as they are degradable. There are many known plants which are sources of such insecticides. Besides being less harmful these do not pose pollution hazards. More than 2000 species of plants have been reported to have insecticidal properties (Morallo-Rejesus and Dece, 1983; Caius, 1986).

Another field of research concerns with plant products having antifeedant/deterrent action (Sumimoto, *et al.*, 1975; Sutherland, *et al.*, 1975; Stermitz and Sharifi, 1977; Adams and Bernays, 1978; Picman, *et al.*, 1978; Smelyanez, 1978; Carpenter, *et al.*, 1979; Nawrot, *et al.*, 1983; Harmatha and Nawrot, 1984; Villani and Gould, 1985b; Nawrot, *et al.*, 1985; Saxena, *et al.*, 1986; Saim and Clifton, 1986; Mohiuddin, *et al.*, 1987). In this context, it is important to note that lot of work has been conducted utilizing various parts and extracts thereof of single species of plant namely, *Azadirachta indica* A. Juss., all over the world by several scientists (Ladd, 1980; Jilani and Helen, 1983; Malik and Naqvi, 1984; Verma and Pandey, 1987). So by using plant products as deterrents of insect pests they could be controlled without causing health hazards as well as disruption of ecological balance.

In the light of above discussion and the observations presented in this work it was decided to undertake further work on leaf extracts found effective against *L. serricorne* F., by applying dosimetaric approach so as to arrive at a better understanding of possibility of practical application of this information. This chapter reports on findings related to five deterrent plant species previously identified during the course of present work.

MATERIAL AND METHODS

Raising the culture of test insect, L. serricorne F., preparing the leaf extracts of as well testing procedures were as described previously (Chapter 2.1).

Four arbitrarily chosen doses were selected in case of all plant leaf extracts — 0.5, 1.0, 2.0, and 5.0 ml of crude extracts. These doses were tested for influence on behaviour of adults and 3rd and 4th larval instars. Each dose was allowed to get soaked up completely in 10 paper discs of 10 mm diameter. After drying at room temperature the repeatedly soaked paper discs were used as "treated" paper discs. Controls were prepared by soaking with respective solvent only and called as "untreated" paper discs.

In case of larval tests, for each desired dose the solution was poured into wheat flour stirring frequently for mixing the quantity with 10 gm of wheat flour. Thereafter the flour samples were dried completely at room temperature and labelled as particular "treated" flour samples after the names of the extracting media. Control samples were made by mixing the respective extracting media with 10 gm of wheat flour in each case. The responses of adult beetles as well as those of 3rd and 4th instar larvae with open thimbles were recorded employing "choice chamber" technique as outlined previously (chapter 2.1). Percentage of deterrency was calculated with respect to different samples by using the following formula:-

$$\% \text{ deterrence} = \frac{(UT + IND)}{Tn} \times 100$$

UT = Number of individuals on untreated sample

IND = Number of individuals showing indifferent

Tn = Total number of individuals released.

RESULTS

The deterrent influence of leaf extracts of 5 different locally available plant species viz. — *Alangium lamarkii*, *Tecoma stans*, *Euphorbia neriifolia*, *Achyranthes aspera* and *Vinca rosea* were assayed by counting the number of individuals found with "treated" or "control" sample or elsewhere showing indifference. The observed results are presented in Tables 1 to 12 and by way of graphs 1 to 6.

It could be clearly seen that (Tables 1 and 2) hot water extract of *A. lamarkii* leaves exhibited very significant deterrent activity against both adults and 3rd and 4th instar larvae. In case of adults (Table 1) raising the dose beyond 2.0 ml did not improve the effectiveness (94%). In case of 3rd instar larvae (Table 2) most effective dose

Table 1. Showing percentage of deterrence on adults to different doses of hot water leaf extract of <u>Alangium lamarkii</u>

Doses	Response shown by individuals to TPD	UTPD	IND	PPR	Deterrence % due to "treated" sample
0.5 ml	08	13	29	16.00 %	84.00 %
1.0 ml	04	13	33	08.00 %	92.00 %
2.0 ml	03	28	19	06.00 %	94.00 %
5.0 ml	03	29	18	06.00 %	94.00 %

TPD = Treated paper discs

UTPD = Untreated paper discs

IND = Column showing indifferent behaviour

PPR = Percentage of positive response to " treated " sample

Table 2. Showing percentage of deterrence on 3rd and 4th instars to different doses of hot water leaf extract of <u>Alangium lamarkii</u>

Doses	Larval instars	Response shown by individuals to TFS	UTFS	IND	PPR	Deterrence % due to "treated" sample
0.5 ml	3rd	11	39	25	14.66 %	85.34 %
	4th	10	32	33	13.33 %	86.67 %
1.0 ml	3rd	12	30	33	16.00 %	84.00 %
	4th	07	26	42	09.33 %	90.67 %
2.0 ml	3rd	15	23	37	20.00 %	80.00 %
	4th	12	29	34	16.00 %	84.00 %
5.0 ml	3rd	17	21	37	22.66 %	77.34 %
	4th	09	24	42	12.00 %	88.00 %

TFS = Treated flour sample

UTFS = Untreated flour sample

IND = Column showing indifferent behaviour

PPR = Percentage of positive response to "treated" sample

Table 3. Showing percentage of deterrence on adults to different doses of hot water leaf extract of *Tecoma stans*

Doses	Response shown by individuals to TPD	UTPD	IND	PPR	Deterrence % due to "treated" sample
0.5 ml	08	26	16	16.00 %	84.00 %
1.0 ml	07	22	21	14.00 %	86.00 %
2.0 ml	04	25	21	08.00 %	92.00 %
5.0 ml	06	23	21	12.00 %	88.00 %

TPD = Treated paper discs

UTPD = Untreated paper discs

IND = Column showing indifferent behaviour

PPR = Percentage of positive response to "treated" sample

Table 4. Showing percentage of deterrence on 3rd and 4th instars to different doses of hot water leaf extract of <u>Tecoma stans</u>

Doses	Larval instars	Response shown by individuals to TFS	UTFS	IND	PPR	Deterrence % due to "treated" sample
0.5 ml	3rd	13	27	35	17.33 %	82.67 %
	4th	12	40	23	16.00 %	84.00 %
1.0 ml	3rd	13	28	34	17.33 %	82.67 %
	4th	23	25	27	30.66 %	69.34 %
2.0 ml	3rd	09	19	47	12.00 %	88.00 %
	4th	14	20	41	18.66 %	81.34 %
5.0 ml	3rd	11	16	48	14.66 %	85.34 %
	4th	25	23	27	33.33 %	66.67 %

TFS = Treated flour sample

UTFS = Untreated flour sample

IND = Column showing indifferent behaviour

PPR = Percentage of positive response to "treated" sample

Table 5. Showing percentage of deterrence on adults to different doses of alkaline leaf extract of Tecoma stans

Doses	Response shown by individuals to TPD	UTPD	IND	PPR	Deterrence % due to "treated" sample
0.5 ml	06	24	20	12.00 %	88.00 %
1.0 ml	06	28	16	12.00 %	88.00 %
2.0 ml	06	27	17	12.00 %	88.00 %
5.0 ml	05	24	21	10.00 %	90.00 %

TPD = Treated paper discs

UTPD = Untreated paper discs

IND = Column showing indifferent behaviour

PPR = Percentage of positive response to "treated" sample

Table 6. Showing percentage of deterrence on 3rd and 4th instars to different doses of alkaline leaf extract of <u>Tecoma stans</u>

Doses	Larval instars	Response shown by individuals to TFS	UTFS	IND	PPR	Deterrence % due to "treated" sample
0.5 ml	3rd	11	26	38	14.66 %	85.34 %
	4th	11	34	30	14.66 %	85.34 %
1.0 ml	3rd	08	24	43	10.66 %	89.34 %
	4th	17	30	28	22.66 %	77.34 %
2.0 ml	3rd	08	22	45	10.66 %	89.34 %
	4th	10	43	22	13.33 %	86.67 %
5.0 ml	3rd	07	27	41	09.33 %	90.67 %
	4th	05	46	24	06.66 %	93.34 %

TFS = Treated flour sample

UTFS = Untreated flour sample

IND = Column showing indifferent behaviour

PPR = Percentage of positive response to "treated" sample

Table 7. Showing percentage of deterrence on adults to different doses of steam-distillation leaf extract of *Euphorbia neriifolia*

Doses	Response shown by individuals to				Deterrence % due to "treated" sample
	TPD	UTPD	IND	PPR	
0.5 ml	08	28	14	16.00 %	84.00 %
1.0 ml	06	30	14	12.00 %	88.00 %
2.0 ml	05	32	13	10.00 %	90.00 %
5.0 ml	05	32	13	10.00 %	90.00 %

TPD = Treated paper discs

UTPD = Untreated paper discs

IND = Column showing indifferent behaviour

PPR = Percentage of positive response to "treated" sample

Table 8. Showing percentage of deterrence on 3rd and 4th instars to different doses of steam-distillation leaf extract of <u>Euphorbia neriifolia</u>

Doses	Larval instars	Response shown by individuals to TFS	UTFS	IND	PPR	Deterrence % due to "treated" sample
0.5 ml	3rd	23	33	19	30.66 %	69.34 %
	4th	09	36	30	12.00 %	88.00 %
1.0 ml	3rd	17	32	26	22.66 %	77.34 %
	4th	16	33	26	21.33 %	78.67 %
2.0 ml	3rd	17	27	31	22.66 %	77.34 %
	4th	09	33	33	12.00 %	88.00 %
5.0 ml	3rd	24	19	22	32.00 %	68.00 %
	4th	11	26	38	14.66 %	85.34 %

TFS = Treated flour sample

UTFS = Untreated flour sample

IND = Column showing indifferent behaviour

PPR = Percentage of positive response to "treated" sample

Table 9. Showing percentage of deterrence on adults to different doses of steam-distillation leaf extract of *Achyranthes aspera*

Doses	Response shown by individuals to				Deterrence % due to "treated" sample
	TPD	UTPD	IND	PPR	
0.5 ml	05	26	19	10.00 %	90.00 %
1.0 ml	04	26	20	08.00 %	92.00 %
2.0 ml	04	24	22	08.00 %	92.00 %
5.0 ml	04	25	21	08.00 %	92.00 %

TPD = Treated paper discs

UTPD = Untreated paper discs

IND = Column showing indifferent behaviour

PPR = Percentage of positive response to "treated" sample

Table 10. Showing percentage of deterrence on 3rd and 4th instars to different doses of steam-distillation leaf extract of *Achyranthes aspera*

Doses	Larval instars	Response shown by individuals to TFS	UTFS	IND	PPR	Deterrence % due to "treated" sample
0.5 ml	3rd	17	36	22	22.66 %	77.34 %
	4th	16	27	32	21.33 %	78.67 %
1.0 ml	3rd	16	28	31	21.33 %	78.67 %
	4th	15	29	31	20.00 %	80.00 %
2.0 ml	3rd	18	29	28	24.00 %	76.00 %
	4th	15	34	26	20.00 %	80.00 %
5.0 ml	3rd	20	29	26	26.66 %	73.34 %
	4th	12	31	32	16.00 %	84.00 %

TFS = Treated flour sample

UTFS = Untreated flour sample

IND = Column showing indifferent behaviour

PPR == Percentage of positive response to "treated" sample

Table 11. Showing percentage of deterrence on adults to different doses of alkaline leaf extract of *Vinca rosea*

Doses	Response shown by individuals to				Deterrence % due to "treated" sample
	TPD	UTPD	IND	PPR	
0.5 ml	09	22	19	18.00 %	82.00 %
1.0 ml	03	22	25	06.00 %	94.00 %
2.0 ml	04	25	21	08.00 %	92.00 %
5.0 ml	03	23	24	06.00 %	94.00 %

TPD = Treated paper discs

UTPD = Untreated paper discs

IND = Column showing indifferent behaviour

PPR = Percentage od positive response to "treated" sample

Table 12. Showing percentage of deterrence on 3rd ahd 4th instars to different doses of alkaline leaf extract of Vinca rosea

Doses	Larval instars	Response shown by individuals to TFS	UTFS	IND	PPR	Deterrence % due to "treated" sample
0.5 ml	3rd	13	30	32	17.33 %	82.67 %
	4th	08	35	32	10.66 %	89.34 %
1.0 ml	3rd	14	39	22	18.66 %	81.34 %
	4th	12	23	40	16.00 %	84.00 %
2.0 ml	3rd	22	21	32	29.33 %	70.67 %
	4th	09	18	48	12.00 %	88.00 %
5.0 ml	3rd	11	34	30	14.66 %	85.34 %
	4th	14	23	38	18.66 %	81.34 %

TFS = Treated flour sample

UTFS = Untreated flour sample

IND = Column showing indifferent behaviour

PPR = Percentage of positive response to "treated" sample

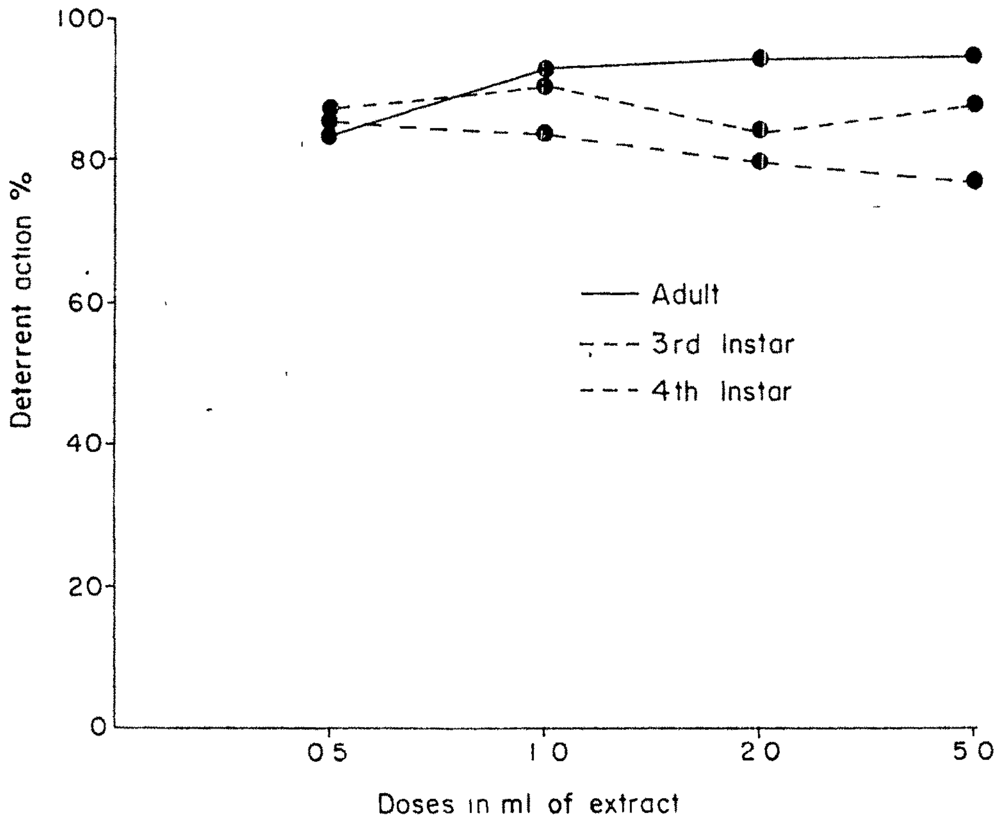

Fig. I Graphic presentation of relationship between deterrent action and dose levels of hot water leaf-extract of the plant Alangium Lamarkii on adult and larval forms of L. serricorne F.

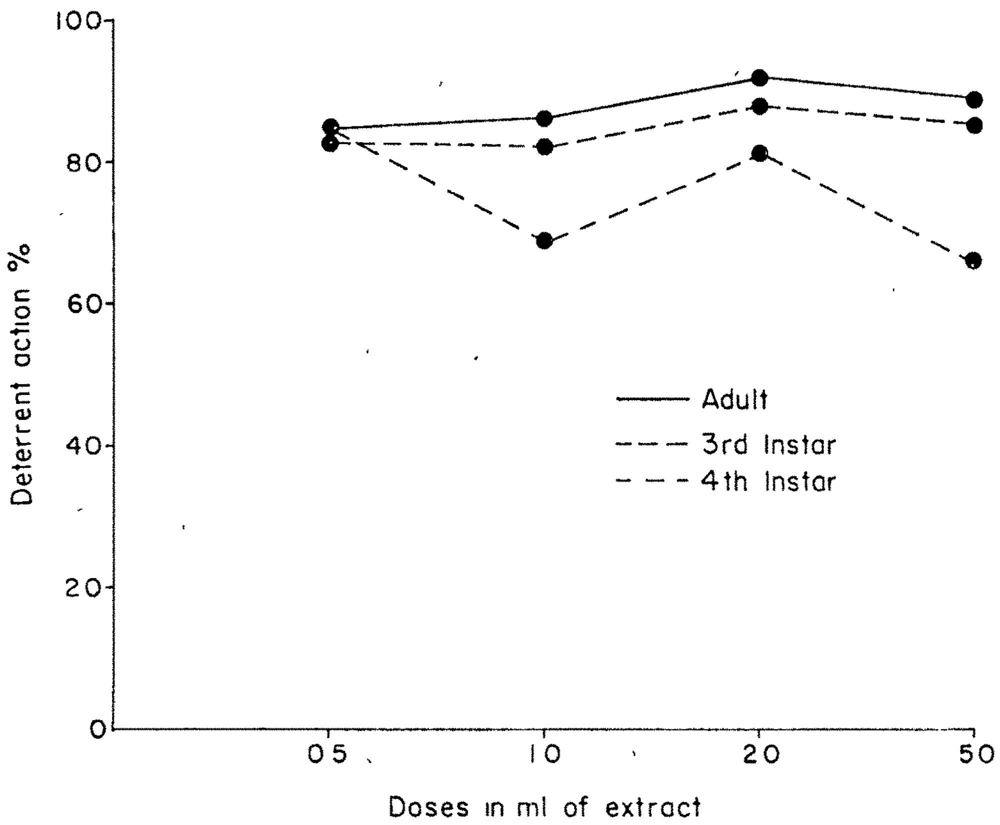

Fig.2 Graphic presentation of relationship between deterrent action and dose levels of hot water leaf-extract of the plant Tecoma stans on adult and larval forms of L. serricorne F.

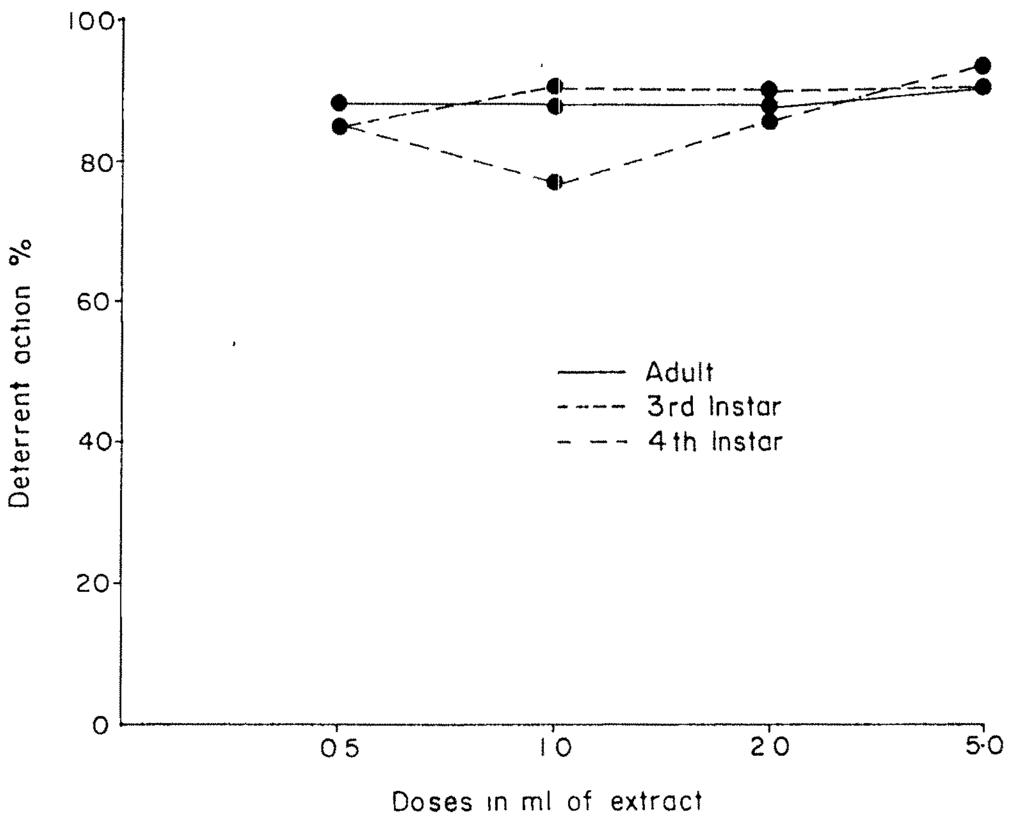

Fig. 3 Graphic presentation of relationship between deterrent action and dose levels of alkaline leaf-extract of the plant Tecoma stans on adult and larval forms of L. serricorne F.

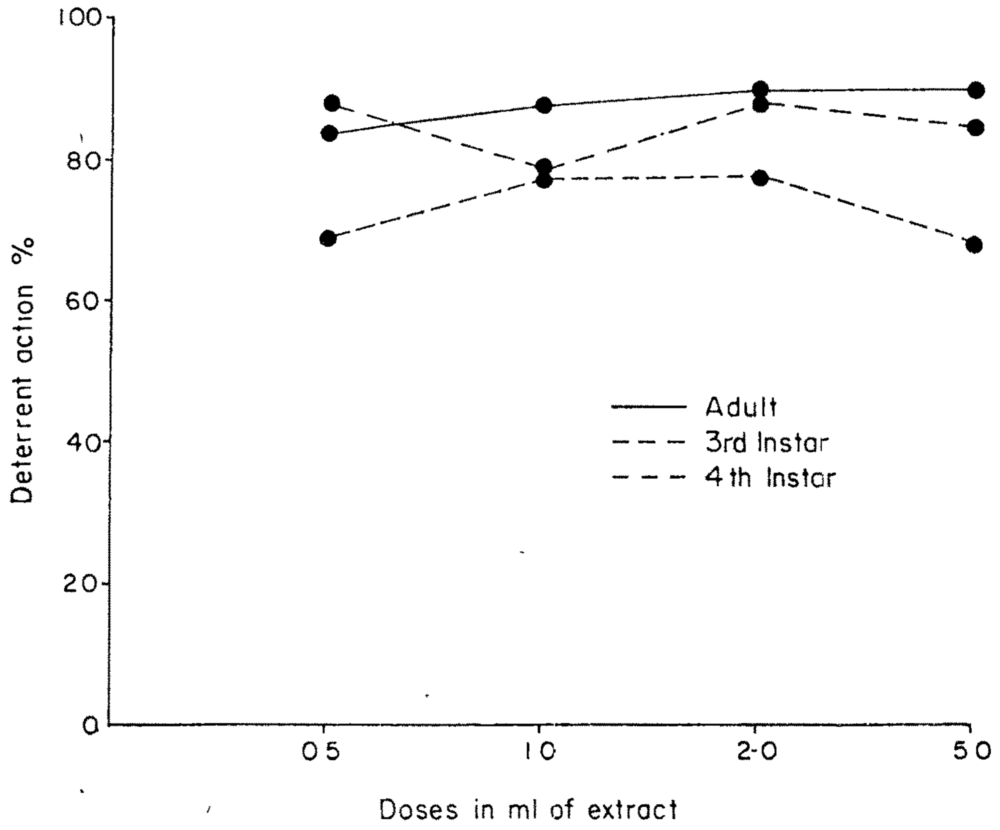

Fig. 4 Graphic presentation of relationship between deterrent action and dose levels of steam-distillation leaf-extract of the plant Euphorbia neriifolia on adult and larval forms of L. serricorne F.

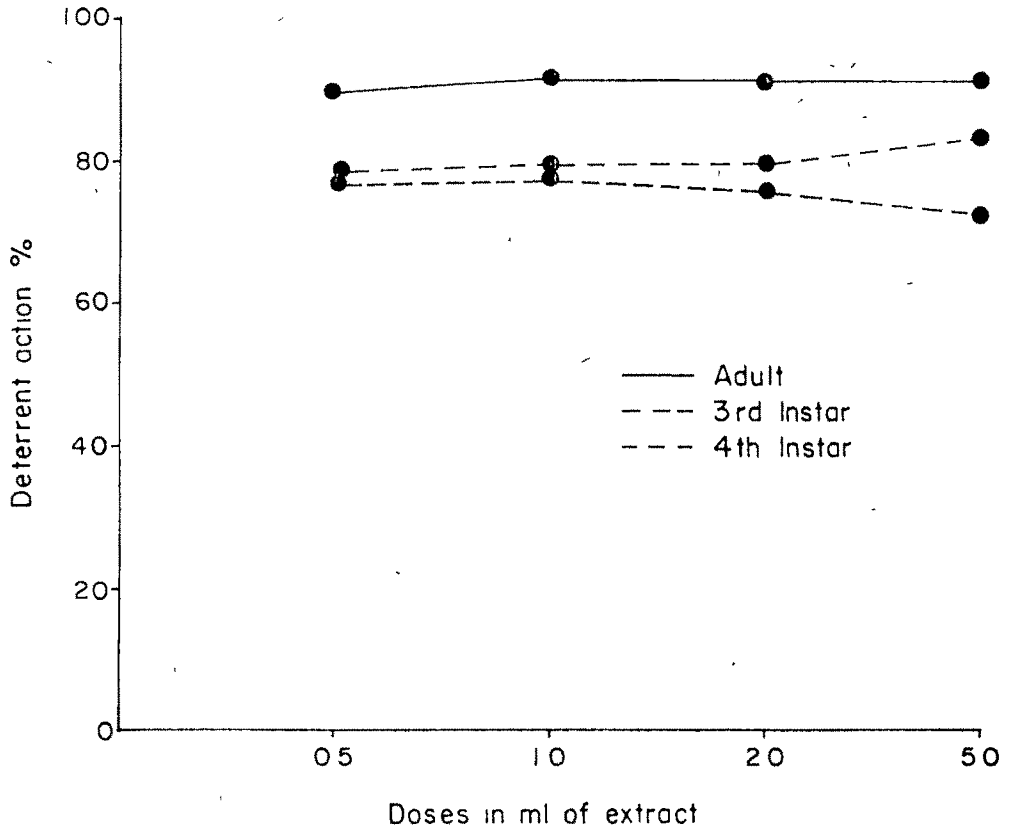

Fig. 5 Graphic presentation of relationship between deterrent action and dose levels of steam - distillation leaf - extract of the plant Achyranthes aspera on adult and larval forms of L. serricorne F.

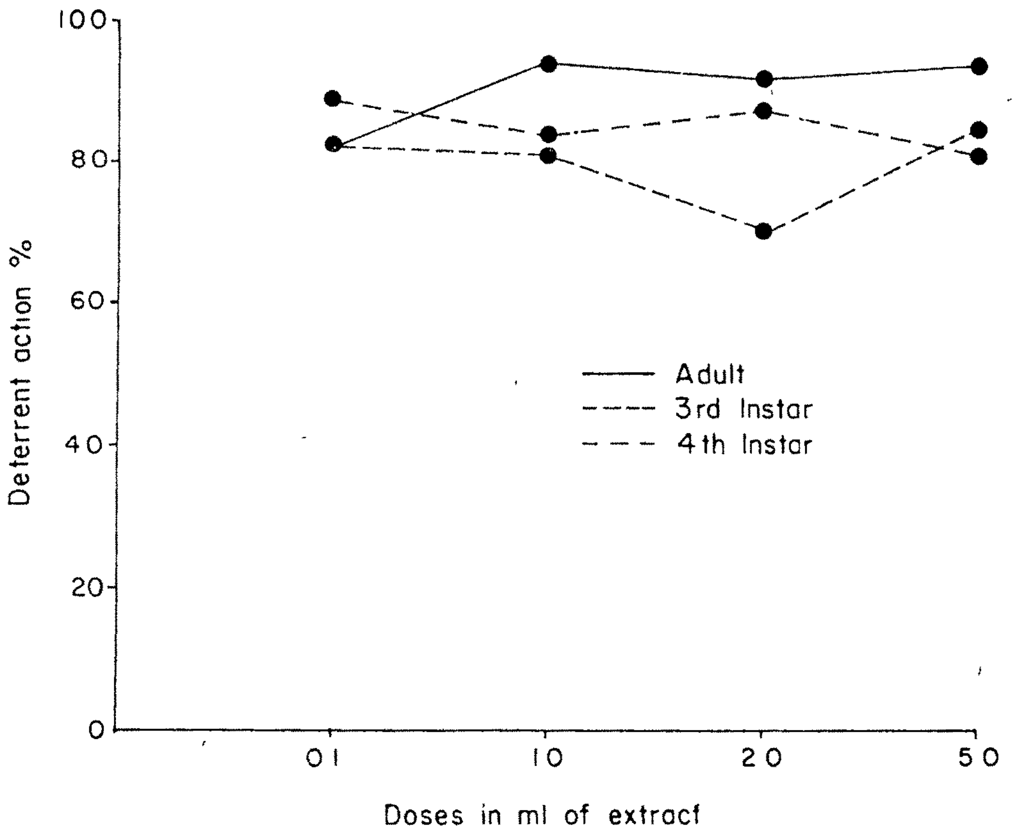

Fig. 6 Graphic presentation of relationship between deterrent action and dose levels of alkaline leaf-extract of the plant Vinca rosea on adult and larval forms of L. serricorne F.

was 0.5 ml (85.34% deterrence). For 4th instar larvae, 1.0 ml dose maximally deterred (90.67%) them. Figure 1 graphically shows composite relationship between the dose levels and larval instars as well as adults.

Data presented on Table 3 and 4 were revealed that the hot water leaf extracts of _T. stans_ exhibited deterrent influence of varying degrees in case of both the adult and larval forms. Perusal of adult responses (Table 3) related a dose-dependent increase in deterring influence upto 2.0 ml dose of crude extract (84% to 92%) beyond which there was no beneficial action. A dose of 2.0 ml (Table 4) was most effective in deterring the 3rd instar larvae (88%). In case of 4th instar larvae the lowest dose of 0.5 ml itself exhibited maximum deterrent action (84%), where as this last instar exhibited fluctuating responses to other doses. This latter variation was probably due to age maturity of this particular instar, which is known to feed very voraciously during early maturity but stops feeding just prior to pupation as a preparatory phase. It is, therefore, likely that in the mixed collection 4th instar, there were some in active feeding phase and other of advanced maturity and about to pupate. The active feeders were probably compelled to ignore even the presence of deterrent, hence the fluctuations. The graphic presentation of composite results are given in Figure 2.

The alkaline leaf extract of the same plant T. stans apparently was better in the sense that with the lowest dose of 0.5 ml itself it could exert deterrent influence, which could not be surpassed to any significant extent with increasing doses, and, remarkably enough in case of both adult beetles as well as larvae (Tables 5 and 6 and Figure 3).

Observations with steam distillate of leaves of E. neriifolia (Table 7) in respect of effective repulsion of adult beetles it was seen that there was a dose-dependent action from 0.5 to 2.0 ml volumes (84% to 90%) but further improvement was not there. From the Table 8 it can be seen that for 3rd instar larvae 1.0 ml dose was effective and in case of the 4th instar larvae the lowest dose 0.5 ml itself was enough. In both the case rise in dose-levels did not lead to any betterment. Graphic representation of all the readings are given in Figure 4.

Influence of the steam distillate of leaves of another plant A. aspera are recorded for both adults and larval forms in Tables 9 and 10. From the Table 9 it was obvious that 1.0 ml dose exerted maximum deterrent action (92%) on adult beetles, which could not improved further by increasing the dose-levels. With respect to larval tests (Table 10) it was noticed that only 0.5 ml dose was enough to exert maximum deterrent influence (77.34%) in the case of 3rd instar. The 4th instar larvae however, exhibited a dose-dependent response within the range 1.0 ml to 5.0 ml of crude extract. Graphic representation of all the readings are given in Figure 5.

The alkaline leaf extracts of the plant V. rosea exhibited excellent deterrent activity against the adult as well as 3rd and 4th instar larvae (Table 11 and 12). The adult beetles were deterred to 94% with 1.0 ml dose that showed no further gain with higher doses (Table 11). In case of both larval instars maximum deterrent activity (Table 12) was recorded with the lowest dose of 0.5 ml only. Obviously the alkaline leaf extract of this commonly growing wild plant species can be very effectively prepared and used by common man to protect the stored food commodities from infestation due to this species. A composite graphic presentation of results are given in figure 6.

DISCUSSION

From a careful perusal of the literature it becomes quite clear that there is wealth of information available on the control of insect pests with the help of different parts of the several plants and a variety of extracts thereof. One of the widely researched single species of plants happens to be the Indian neem plant — Azadirachta indica A. Juss. The leaves, tender twigs, seeds, seed powder, kernel extracts, etc. of neem plant have been utilized towards this purpose. Azadirachtin, oil of Margosa and powder seed cake are the subject of interest even to date. Similarly, many other plant species have been scanned for antifeedant/ deterrent influences, derievable from roots to the seeds, have been tackled. In this context, the present chapter has dealt with only the leaf extracts of 5 species of plants, which were found to possess deterrent properties, on the basis of observations reported in early chapters (2.1 and 2.2).

As regards the methods of extraction employed by different workers, it can be said that petroleum ether, acetone and ethanol have been the solvents of common choice. Petroleum ether extraction of the seeds of 50 different plants to extract possible repellent/antifeedant factors has been employed by Khan et al.,(1983). On the other hand, Pandey et al.,(1986) have utilized petroleum ether extraction and subsequent dilution with benzene of several plants and their different parts for repulsive components. Acetone extraction of seed oils of Coriandrum sativum has been employed by Su, Helen (1984a and 1986), for repellent activity against insect pests. Reed et al.,(1982) isolated azadirachtin and salanin as ethanolic extracts of neem seeds for demonstrating feeding deterrent influence.

During the course of the present work also petroleum ether and acetone extraction has been used along with some other solvents like methanol and chloroform-methanol (2 : 1). Additionally, hot water and alkaline extraction of leaves and also steam-distillation were tried out.

General methods of application of such extracts have been through rubbing them on grains before storage, spraying on the stored products, using paper strips soaked with extracts and plain mixing. Presently, the filter paper discs soaked in the extracts and mixing the latter with wheat flour were the methods of choice, which were in keeping with above mentioned trends. Further, as has been reported by several authors (cited above) here also observations on varying doses of the extracts under investigation have been carried out in order to find the minimal possible doses exerting maximal deterrence.

Ivbijaro (1983) showed that powdered neem seed at the dose levels of 0.5, 1.0, and 2.5 gm/20 gm mixed with maize prevented infestation by Sitophilus oryzae. Su, Helen (1984b) has successfully used pulvarized pericarp of Zanthozylum alatum Roxb. fruits at 0.5, 1.0 and 2.0% levels to deter significantly the Sitiphilus oryzae from infestation of stored wheat grains. With surface treatment of wheat grains at doses of 0.05, 0.1 and 0.2% with the seed oil of Coriandrum sativum L. has been shown to provide protectant action against S. oryzae as well as Tribolium castaneum (Su,Helen,1986). Using turmeric oil at 100, 500 and 1000 ppm Jilani et al.,(1988) have shown that Tribolium castaneum Herbst. could be repelled most effectively.

In the light of above information, and, also based on the few results at hand, one can suggest by way of tentative conclusion, that alkaline extracts of V. rosea and T. stans are probably the best agents for deterring away, with the minimum dose levels, most of the stages of the life cycle of L. serricorne F. Moreover, for a common man, preparing an alkaline extract of the leaves of these very common and widespread species of Indian plants, is not a difficult procedure for practical application. Further, very low doses of these extracts were highly effective deterrents for both larval as well as adults forms. It is, therefore, highly desirable to attempt to isolate the most effective components of such extracts by employing suitable methods of chemical separation. Some information on this aspect is given in chapter 4.1. However, it should be pointed out here that a lot of further work on toxicological properties need be done before this information can be transformed into a practical method of management of L. serricorne F., a serious pest, not only in cured tobacco godowns, but also of a considerable variety of stored food products, including cereal grains.

SUMMARY

5 different plant leaf extracts viz.— A. lamarkii, T. stans, E. neriifolia, A. aspera, and V. rosea were tested by "choice chamber" technique for their deterrent influence on the adults and 3rd and 4th instar larvae of cigarette beetle, L. serricorne F. Four arbitrarily selected doses viz. — 0.5, 1.0, 2.0, and 5.0 ml of crude extracts were tested. Hot water leaf extract of A. lamarkii plant exhibited 94% repulsion with 2.0 ml dose of adults, whereas with only 1.0 ml dose 3rd and 4th instar larvae exhibited 84% and 90.67% reduction in feeding activity respectively. 2.0 and 5.0 ml of hot water and alkaline extracts of another plant T. stans were most effective against adults as well as the larval stages. Of the two extracts the alkaline one inhibited the larvae more than the hot water extract. Steam distillate of A. aspera leaves exhibited promising results against the adults (92% repulsion), but the larvae were not influenced to that extent. Steam distillate of E. neriifolia could deter the adults to 90% but only at a higher dose of 2.0 ml. In contrast to this a dose of 0.5 ml effectively inhibited 88% of the 4th instar larvae. Another very common medicinal plant in V. rosea leaves in alkaline extract at 1.0 ml dose exhibited 94% repulsion of adults. A 0.5 ml dose of same was found to exert 89.34% of deterrence in the case of the 4th instar

larvae of *L. serricorne* F. On the basis of these observa - tions it has been suggested that the alkaline extracts of *T. stans* and *V. rosea* are the most suitable deterrents of both larval as well as adult stages of this pest. Further, it has also been pointed out that unless toxical properties are studied the information should not be utilized for practical application.

CHAPTER 3.2

DOSIMETRIC STUDIES ON ARRESTANT INFLUENCE OF LEAF EXTRACTS OF THREE OTHER PLANT SPECIES ON ADULT AND LARVAL FORMS OF CIGARETTE BEETLE, LASIODERMA SERRICORNE F.

Cigarette beetle, *Lasioderma serricorne* F. is the serious pest of our stored commodities. It would be useful if it could be controlled by devising arrestant baits with the help of biodegreadable non-toxic plant materials. For several years liquid with arrestant quality in the form of baits as soaked cotton wicks have been used to trap the Japanese beetle, *Popillia japonica* (Newman.) (Metzger, 1933; Fleming, 1969; Goonewardene, 1970). Foott and Hybsky (1976) have suggested trapping beetles to control their populations. Much work has been done regarding the attraction of insect to light and light traps (Cantelo, 1974; Hienton, 1974). According to Keever and Daniel (1983) most studies on the responses of stored product insect pests to light have dealt more with basic topics, such as spectral response, rather than applied trapping techniques. Ladd *et al.*, (1983) reported that eugenol would attract adult northern corn rootworm, *Diabrotica barberi* Smith and Lawrence. This appears to be the 1st instance in which a pure, nonpheromonal compound not specifically related to the host plant, has been so described. Mixtures of chemicals have been recommended as attractants for the Japanese beetle, P. japonica N. (McGovern and Ladd, 1984). It is surprising to note that only very few reports are available on this interesting aspect of trapping the stored-product pest beetles. In this context, the present work report on the potential of plant products as baits to attract a serious pest L. serricorne F., would prove to be an economical and safer way of management of this pest. The current work deals with the leaf extracts of three common Indian plant species as attractants. Dosimetric observations on simple crude extracts have been made so as find out minimum effective dose levels with an eye on possible future applicability for protection of stored products from infestation by L. serricorne F.

MATERIAL AND METHODS

All the details of experimental methods have already been outlined in the chapter 3.1, except that the percentage of arrestant action was calculated by employing the following formula :-

Arrestant action expressed as % = $\frac{T}{Tn}$ X 100

(number of individuals on the bait)

where T = Number of individual on "treated" bait,

Tn = Total number of individuals released

RESULTS

During the course of present investigation the leaf extracts of three different plant species _viz._— _Ocimum sanctum_, _Ipomoea fistulosa_, and _Datura matel_ were tested for arrestant influence at the dose-levels— 0.5, 1.0, 2.0, and 5.0 ml of crude extracts. Responses of the adult as well as 3rd and 4th instar larvae are recorded in the Tables 1 to 6 and graphically represented in Figures 1 to 3.

From Table 1 it could be clearly seen that the chloroform-methanolic leaf extract of the plant _O. sanctum_ exhibited a dose-dependent arrestant action on adult insects (60% to 84%). In case of larvae (Table 2) it was clear that 4th instar larvae were arrested to a maximum level of 60% by the lowest dose of 0.5 ml only. Higher doses were not only superfluous but exerted aberrant influence, hence raising the dose is not advisable. The 3rd instar larvae, on the other hand, could be arrested to a maximum

Table 1. Showing percentage of arrestant action on adults to different doses of chloroform-methanol leaf extract of *Ocimum sanctum*

Doses	Response shown by individuals to		IND	Arrestant action % due to "treated" sample
	TPD	UTPD		
0.5 ml	33	13	04	66.00 %
1.0 ml	36	14	00	72.00 %
2.0 ml	37	09	04	74.00 %
5.0 ml	42	07	01	84.00 %

TPD = Treated paper discs

UTPD = Untreated paper discs

IND = Column showing indifferent behaviour

Table 2. Showing percentage of arrestant action on 3rd and 4th instars to different doses of chloroform-methanol leaf extract of <u>Ocimum sanctum</u>

Doses	Larval instars	Response shown by individuals to			Arrestant action % due to "treated" sample
		TFS	UTFS	IND	
0.5 ml	3rd	36	13	26	48.00 %
	4th	45	13	17	60.00 %
1.0 ml	3rd	38	11	26	50.67 %
	4th	44	15	16	58.67 %
2.0 ml	3rd	40	13	22	53.34 %
	4th	45	13	17	60.00 %
5.0 ml	3rd	36	11	28	48.00 %
	4th	40	20	15	53.34 %

TFS = Treated flour sample

UTFS = Utreated flour sample

IND = Column showing indifferent behaviour

Table 3. Showing percentage of arrestant action on adults to different doses of hot water leaf extract of <u>Ipomoea fistulosa</u>

Doses	Response shown by individuals to TPD	UTPD	IND	Arrestant action % due to "treated" sample
0.5 ml	28	12	10	56.00 %
1.0 ml	30	14	06	60.00 %
2.0 ml	25	17	08	50.00 %
5.0 ml	28	12	10	56.00 %

TPD = Treated paper discs

UTPD = Untreated paper discs

IND = Column showing indifferent behaviour

Table 4. Showing percentage of arrestant action on 3rd and 4th instars to different doses of hot water leaf extract of <u>Ipomoea fistulosa</u>

Doses	Larval instars	Response shown by individuals to		IND	Arrestant action % due to "treated" sample
		TFS	UTFS		
0.5 ml	3rd	28	17	30	37.34 %
	4th	38	13	24	50.67 %
1.0 ml	3rd	38	17	20	50.67 %
	4th	32	14	29	42.67 %
2.0 ml	3rd	37	16	22	49.34 %
	4th	37	15	23	49.34 %
5.0 ml	3rd	34	16	25	45.34 %
	4th	36	20	19	48.00 %

TFS = Treated flour sample

UTFS = Untreated flour sample

IND = Column showing indifferent behaviour

Table 5. Showing percentage of arrestant action on adults to different doses of alkaline leaf extract of *Datura matel*

Doses	Response shown by individuals to TPD	UTPD	IND	Arrestant action % due to "treated" sample
0.5 ml	16	20	14	32.00 %
1.0 ml	24	16	10	48.00 %
2.0 ml	29	15	06	58.00 %
5.0 ml	27	14	09	54.00 %

TPD = Treated paper discs

UTPD = Untreated paper discs

IND = Column showing indifferent behaviour

Table 6. Showing percentage of arrestant action on 3rd and 4th instars to different doses of alkaline leaf extract of <u>Datura matel</u>

Doses	Larval instars	Response shown by individuals to TFS	UTFS	IND	Arrestant action % due to "treated" sample
0.5 ml	3rd	36	17	22	48.00 %
	4th	28	31	16	37.34 %
1.0 ml	3rd	36	21	18	48.00 %
	4th	33	20	22	44.00 %
2.0 ml	3rd	32	20	23	42.67 %
	4th	28	19	28	37.34 %
5.0 ml	3rd	22	30	23	29.34 %
	4th	22	28	25	29.34 %

TFS = Treated flour sample

UTFS = Untreated flour sample

IND = Column showing indifferent behaviour

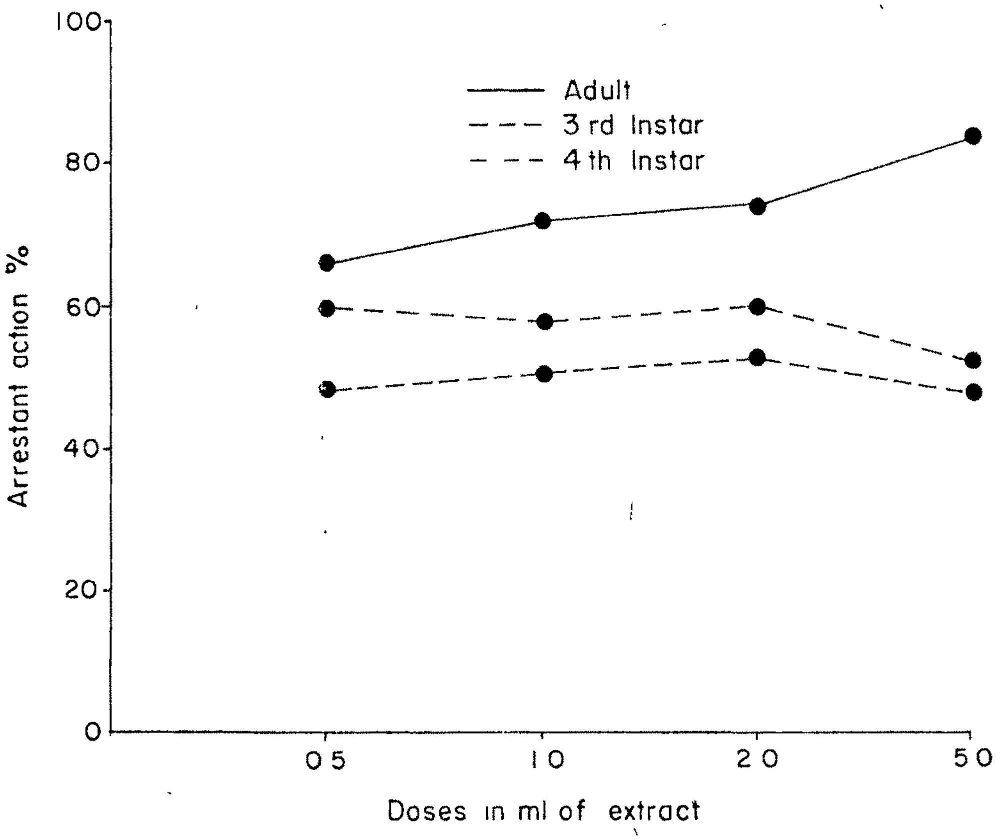

Fig. I Graphic presentation of relationship between arrestant action and dose levels of chloroform - methanolic leaf - extract of the plant <u>Ocimum sanctum</u> on adult and larval forms of <u>L</u>. <u>serricorne</u> F.

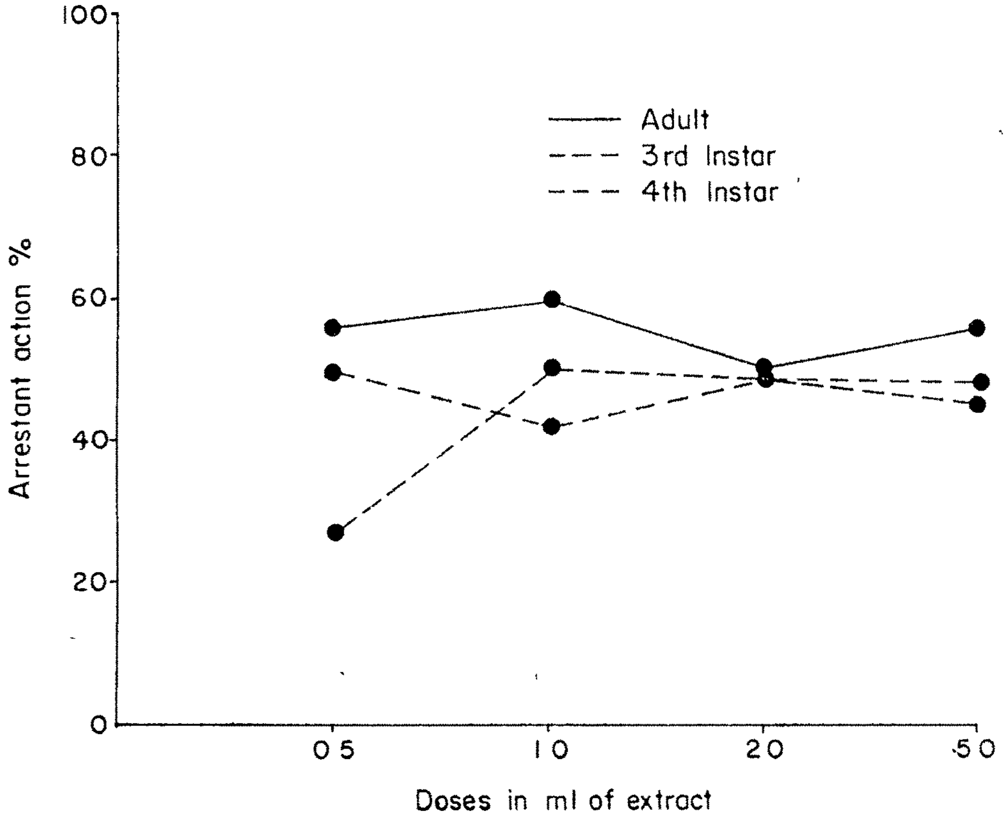

Fig. 2 Graphic presentation of relationship between arrestant action and dose levels of hot water leaf-extract of the plant Ipomoea fistulosa on adult and larval forms of L. serricorne F.

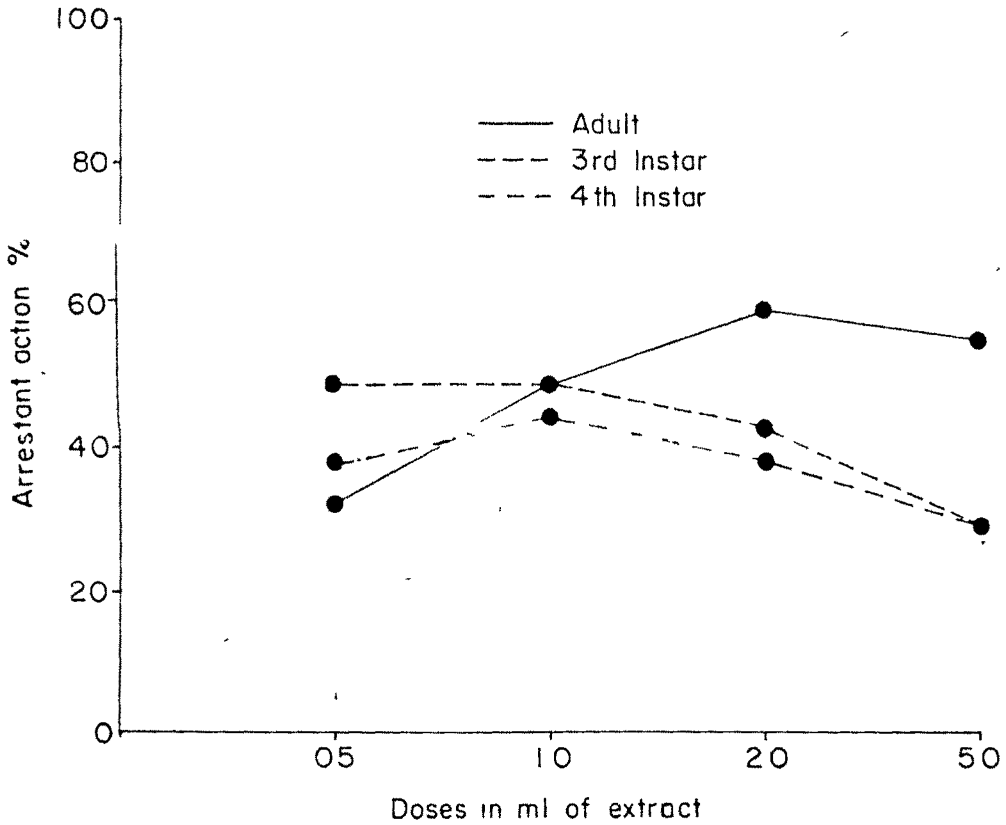

Fig. 3 Graphic presentation of relationship between arrestant action and dose levels of alkaline leaf-extract of the plant <u>Datura</u> <u>matel</u> on adult and larval forms of <u>L.</u> <u>serricorne</u> F.

extent in their movements only by as high a dose as 2 ml. The composite representation of arrestant actions is also shown in Figure 1 graphically.

Data presented in Tables 3 and 4 show the arrestant activity of hot water leaf extracts of the plant I. fistulosa on the adults and 3rd and 4th instar larvae with the 4 selected doses. In the case of this particular leaf extract it is possible to generalize that at 1.0 ml dose all the stages of this insect species were arrested in the range of 50% to 60% only. The composite representation of arrestant actions is also shown in Figure 2 graphically.

A statement similar to above one is also applicable in case of another common plant, D. matel. The alkaline extract elicited an arrestant influence with 1.0 to 2.0 ml doses against adults (Table 5) as well as larvae (Table 6) in the range of 48% to 58% only. Figure 3 indicates graphically the influence on both adult and larval forms.

DISCUSSION

Though in the course of preliminary studies it was noted the leaves of three particular plant species chosen here were effective arrestants for L. serricorne F., the dosimetric studies reported in this chapter here clearly demonstrated that but for O. sanctum the other two plants were not satisfactory candidates for further investigation. The chloroform-methanol leaf extract from O. sanctum was capable of exerting more than 80% arrestant action on adults and also an appreciable action on larval forms.

In this context, the work of Kamm and Buttery (1984) is worth noting. They reported that the root volatile components of the red clover (Trifolium pratense) were attractive to the clover root borer (Hylastinus obscurus).

Stubbs et al.,(1985) have reported that the vacuum distillation of heat treated carob (Ceratonia silique) gave an aqueous, colourless, sweet smelling distillate which was found to be highly attractive to adult Oryzaephilus surinamensis L. The isolated materials responsible for the attraction were acetic, isobutyric, N-butyric, 2-methyl butyric and hexanoic acids, and that hexanoic acid was the most attractive longer lasting component of carob distillate.

Peacock et al.,(1984) used the multilure baited traps with a bolt of healthy american elm plant, Ulmus americana to attract Scolytus multistriatus. Traps baited only with multilure were less effective but those with elm bolt combination were 2 to 3 times more attractive and 4 to 18 times so as bolt alone. The increased attraction from the presence of healthy elm tissue suggested that additional host volatiles, other than those formulated in multilure, contribute to the aggregation of beetles.

Taking into account the above cited work, it would be interesting to try out all possible combinations of the different leaf extracts studied here in order to obtain better combinations for attracting the species of pest under investigation. It also remains to be investigated whether other methods of extraction would improve the arrestant action of O. sanctum leaves. Further, other parts of this plant need also be tried out.

SUMMARY

Leaves of three different plants, O. sanctum, I. fistulosa, D. matel were extracted in chloroform-methanol mixture, hot water and alkaline solution, respectively for determination of arrestant properties against the adult as well as 3rd and 4th instar larvae of L. serricorne F. Chloroform-methanol leaf extract of O. sanctum exhibited 84% arrestant action at 5.0 ml dose against adult and 60% arrestant activity on only the 4th instar larvae. Other two plants, I. fistulosa and D. matel were showing more or less similar insignificant results against both the adult and larval forms. Possible implications of these findings, in the light of available sparse literature, are discussed.

CHAPTER 3.3

DOSIMETRIC STUDY OF ARRESTANT INFLUENCE OF EXTRACT OF DURANTA PLUMIERI FRUITS (DRIED) AND THAT OF SEEDS OF OCIMUM SANCTUM ON ADULT AND LARVAL FORMS OF LASIODERMA SERRICORNE F.

Much work has been done regarding trapping by attraction of insect pests, related to field crops as well as varieties of stored products. Most of such monitoring efforts have involved the lepidopteran species, but a few of coleopteran species have also been experimented (Barrett, *et al.*, 1971). Traps containing attractants were used to monitor insect pest populations. Female sex pheromones have often been utilized as trap baits (Ball and Chaudhury, 1973; Guss, 1976; Bartelt and Chiang, 1977 and Guss, *et al.*, 1982). Feeding stimulants and attractants have been reported from corn seed, corn kernels and cucurbits (Derr, *et al.*, 1964; Guerra and Shaver, 1969; Howe and Rhodes, 1976 and Metcalf, 1979). Kohno *et al.* (1983) reported the preference tests of the adult cigarette beetle, *Lasioderma serricorne* F. for various host foods and found that the females were strongly attracted to the cured tobacco leaves, but the males were not. On the other hand, Mikolajczak *et al*.,(1984) have explained on the basis of laboratory pitfal bioassay chamber test that the saw-toothed grain beetle, *Oryzaephilus surinamensis* Linn. (Coleoptera : Cucujidae) is attracted to certain volatile components occuring in whole as well as rolled oats. From the above facts it is clear that in recent years most of the workers have tried to avoid use of insecticides for controlling the pest populations either in the fields or under storage conditions, as the use of latter for controlling pest populations usually is not desirable due to their toxic and residual activities. Considerable progress, therefore, has been made in the direction of developing attractants of plant origins, including leaves, flowers, seeds, etc. Buttery *et al*.,(1984) isolated volatile components from red clover (*Trifolium pratense*) leaves, flowers and seeds, which were found to be the possible insect attractants. Buttery and Louisa (1985) reported that volatile components of corn roots also were possible insect attractants. The maize weevil, *Sitophilus zeamais* Motschuslsky., is a cosmopolitan pest of stored grains, especially of corn, *Zeamais* L. and is attracted to some extracts derived from corn (Tipping *et al*., 1986 and 1987). In the light of above-cited literature, it was thought worthwhile to look for some plant products that could be attractants/arrestants in case of *L. serricorne* F., a wide-spread pest of stored cured tobacco leaves in particular and of several other foods and food products under storage, in general. The present work was undertaken on the basis of the results obtained previously (Chapter 2). After having noted that extracts of whole dried fruits of *D. plumieri* and seeds of *O. sanctum*, it was decided to extend this observation by conducting a dosimatric study of these extracts, so as to find out the potentiality for practical application.

MATERIAL AND METHODS

All details were essentially similar to those outlined in the Chapter 3.2 except for the testing materials (fruit and seed extracts).

RESULTS

Methanolic extract of whole dried fruits of the plant *Duranta plumieri* and petroleum ether extract of seeds of the plant *Ocimum sanctum* when tested by choice chamber bioassay methods against the adults as well as 3rd and 4th instar larvae of *L. serricorne* F., (Tables 1 to 4) showed encouraging results only in the former case. With *D. plumieri*

Table 1. Showing percentage of arrestant action on adults to four different doses of methanolic extract of whole dried fruits of <u>Duranta plumieri</u>

Doses	Response shown by individuals to		IND	Arrestant action % due to "treated" sample
	TPD	UTPD		
0.5 ml	33	03	14	66.00 %
1.0 ml	37	02	11	74.00 %
2.0 ml	48	00	02	96.00 %
5.0 ml	47	01	02	94.00 %

TPD = Treated paper discs

UTPD = Untreated paper discs

IND = Column showing indifferent behabiour

Table 2. Showing percentage of arrestant action on 3rd and 4th instars to four different doses of methanolic extract of whole dried fruits of <u>Duranta plumieri</u>

Doses	Larval instars	Response shown by individuals to TFS	UTFS	IND	Arrestant action % due to "treated " sample
0.5 ml	3rd	53	06	16	70.67 %
	4th	46	15	14	61.34 %
1.0 ml	3rd	56	08	11	74.67 %
	4th	54	07	14	72.00 %
2.0 ml	3rd	63	07	05	84.00 %
	4th	66	04	05	88.00 %
5.0 ml	3rd	64	06	05	85.34 %
	4th	61	04	10	81.34 %

TFS = Treated flour sample

UTFS = Untreated flour sample

IND = Column showing indifferent behaviour

Table 3. Showing percentage of arrestant action on adults to four different doses of petroleum ether extract of seeds of <u>Ocimum sanctum</u>

Doses	Response shown by individuals to TPD	UTPD	IND	Arrestant action % due to "treated " sample
0.5 ml	20	17	13	40.00 %
1.0 ml	19	18	13	38.00 %
2.0 ml	17	20	13	34.00 %
5.0 ml	25	14	11	50.00 %

TPD = Treated paper discs

UTPD = Untreated paper discs

IND = Column showing indifferent behaviour

Table 4. Showing percentage of arrestant action on 3rd and 4th instars to four different doses of petroleum-ether extract of seeds of <u>Ocimum sanctum</u>

Doses	Larval instars	Response shown by individuals to TFS	UTFS	IND	Arrestant action % due to "treated" sample
0.5 ml	3rd	24	21	30	32.00 %
	4th	26	25	24	34.67 %
1.0 ml	3rd	22	20	23	29.34 %
	4th	21	26	28	28.00 %
2.0 ml	3rd	25	27	23	33.34 %
	4th	23	20	32	30.67 %
5.0 ml	3rd	22	24	29	29.34 %
	4th	23	19	33	30.67 %

TFS = Treated flour sample

UTFS = Untreated flour sample

IND = Column showing indifferent behaviour

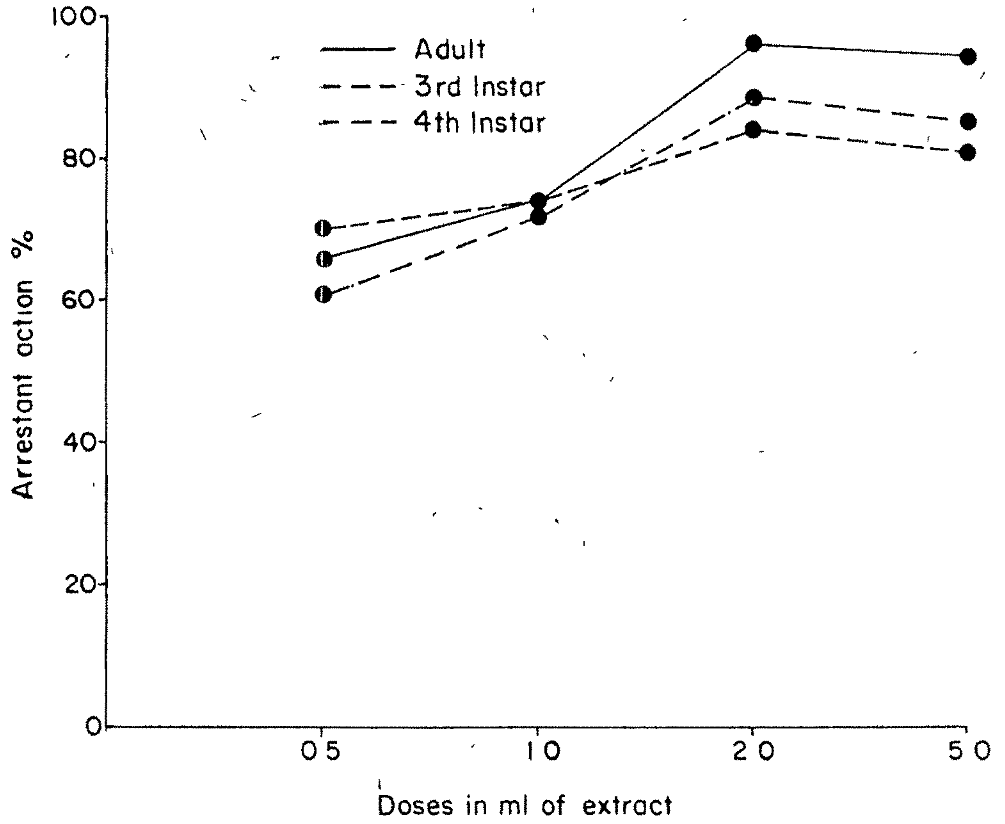

Fig. I Graphic presentation of relationship between arrestant action and dose levels of methanolic extract of dried fruits of the plant Duranta plumieri on adult and larval forms of L. serricorne F.

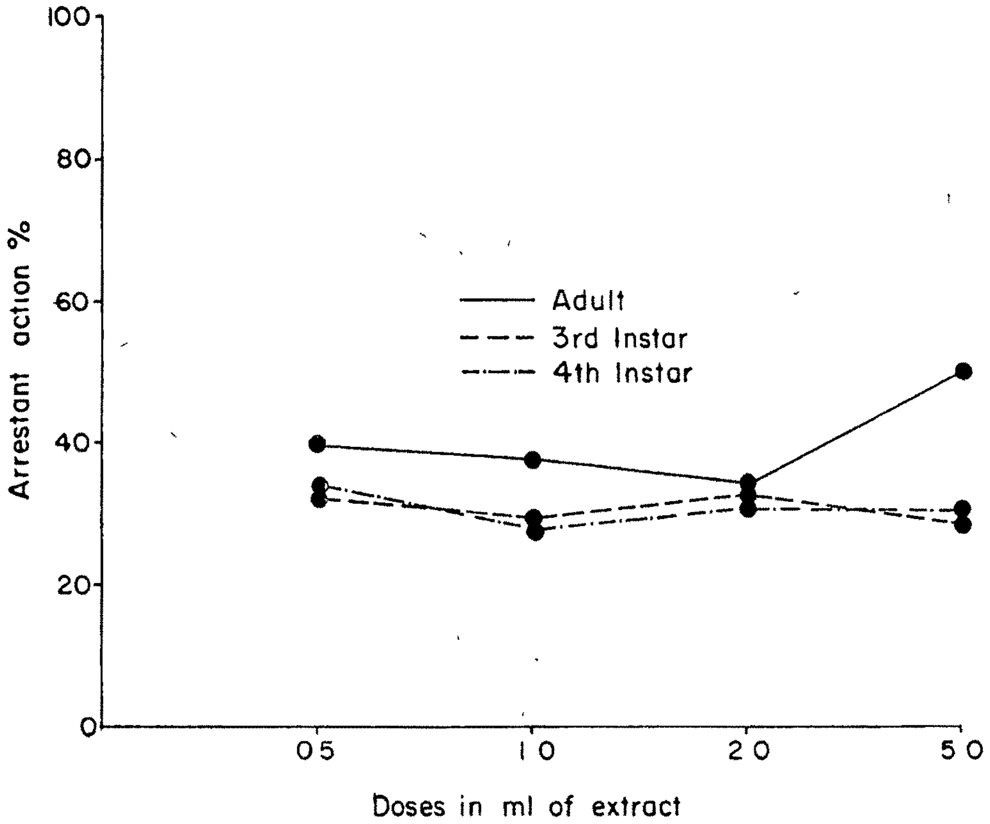

Fig. 2 Graphic presentation of relationship between arrestant action and dose levels of petroleum–ether extract of seeds of the plant Ocimum sanctum on adult and larval forms of L. serricorne F.

extract distinct dose-dependent attractive-arrestant influence was obvious in case of both the adults (66% to 96%) and 3rd and 4th larval instars (70%to 84% and 61% to 88%, respectively) within the dose-range of 0.5 ml to 2.0 ml. As against this, the *O. sanctum* seed extract was observed to exert less than 50% overall attraction even with the highest dose of 5.0 ml. Composite graphic representations of the results are given in Figures 1 and 2.

DISCUSSION

The *D. plumieri* fruit extract exhibited a promising arrestant property upto 96% with 2.0 ml dose. From the work of O'Donnell *et al.* (1983) on attractant properties of four solvent extracts of pods of the carob tree (*Ceratonia siliqua* Linn.) on saw-toothed grain beetle, *Oryzaephilus surinamensis* L., it can be seen that the least polar extract was the most active. These authors have also observed that fractionation of the extracts yielded a series of components, the most attractive of which contained a mixture of triglycerides with 3 or more double bonds per-molecule.

During the course of present study also an attempt has been made at fractionation by employing T.L.C. for chemical components responsible for arrestant action, which are included in Chapter 4.2.

Work carried out by Saxena and Rembold (1984) on 1st and 6th instar larvae of *Heliothis armigera* H. for demonstration of an attractant contained in chick-pea (*Cicer arietinum*) seed powder has proved that a chemical called Kairomonus contained in seed powder is the strong attractant. This attractant was shown to soluble in N-haxene. Present author has also tried *O. sanctum* seed petroleum ether extract but that was found not to contain any significant attractant components. Probably, some other solvents/solvent systems may prove to be of use. Hence, the author wishes to suggest that *D. plumieri* whole dried fruit extract deserves more work.

SUMMARY

The arrestant responses of adults and 3rd and 4th instar larvae of *L. serricorne* F. were evaluated through 4 different doses of the methanolic fruit (*D. plumieri*) extract and petroleum-ether seed extract by employing choice chamber technique. *D. plumieri* fruit extracts exhibited excellent arrestant action for both the adults and 3rd and 4th instar larvae. But *O. sanctum* seed extract does not show any arrestant responses worth notice. In adult tests, *D. plumieri* fruit extract exhibited 96% attraction and in case of larval forms 84% to 88% with 2.0 ml dose.